Blood groups and malaria

Curr Opin Hematol. Author manuscript; available in PMC 2010 May 29.

Published in final edited form as:
Curr Opin Hematol. 2009 November ; 16(6): 480–487. doi:10.1097/MOH.0b013e3283313de0.

Blood groups and malaria: fresh insights into pathogenesis and identification of targets for intervention

J. Alexandra Rowe[a], **D. Herbert Opi**[a,b], and **Thomas N. Williams**[b,c,d]

[a]Centre for Immunity, Infection and Evolution, Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, UK

[b]Centre for Geographic Medicine Research-Coast, Kilifi, Kenya

[c]Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK

[d]Department of Paediatrics, University of Oxford, Oxford, UK

Abstract

Purpose of review—This review summarizes recent advances in our understanding of the interaction between malaria parasites and blood group antigens and discusses how the knowledge gleaned can be used to target the development of new antimalarial treatments and vaccines.

Recent findings—Studies of the interaction between *Plasmodium vivax* and the Duffy antigen provide the clearest example of the potential for basic research on blood groups and malaria to be translated into a vaccine that could have a major impact on global health. Progress is also being made in understanding the effects of other blood group antigens on malaria. After years of controversy, the effect of ABO blood groups on falciparum malaria has been clarified, with the non-O blood groups emerging as significant risk factors for life-threatening malaria, through the mechanism of enhanced rosette formation. The Knops blood group system may also influence malaria susceptibility, although conflicting results from different countries mean that further research is required. Unanswered questions remain about the interactions between malaria parasites and other blood group antigens, including the Gerbich, MNS and Rhesus systems.

Summary—The interplay between malaria parasites and blood group antigens remains a fascinating subject with potential to contribute to the development of new interventions to reduce the global burden of malaria.

Keywords

ABO; Duffy; Knops; plasmodium; virulence

Introduction

Although the study of blood group antigens and malaria parasites is decades old, new advances continue to be made that profoundly influence our understanding of how malaria parasites interact with their human hosts. Because malaria parasites spend a substantial part of their life cycle invading red blood cells (RBCs) and growing within them (Fig. 1) [1], they have evolved specific receptor–ligand interactions to facilitate RBC binding, some of which involve blood group antigens. Variant RBCs with blood group polymorphisms or null

Correspondence to Dr Jane Alexandra Rowe, Institute of Immunology and Infection Research, King's Buildings, University of Edinburgh, West Mains Rd, Edinburgh EH9 3JT, UK Tel: +44 131 6505492; Alex.Rowe@ed.ac.uk.

Europe PMC Funders Author Manuscripts

phenotypes have been used to probe RBC–parasite interactions *in vitro*, and genetic epidemiological studies investigating the effect of blood group polymorphisms on malaria severity have been used to identify molecules and pathways that play a crucial role in life-threatening malaria. Recent advances in some of the major blood group systems affecting malaria are outlined below, followed by important unanswered questions and associations that require further study.

The Duffy blood group

The Duffy blood group antigen provides the clearest example of a malaria resistance mechanism yet described. Its critical role in the invasion of RBC by both the simian parasite *Plasmodium knowlesi* and the related human parasite *P. vivax* was first demonstrated in the mid-1970s [2,3] and subsequently the role of the Duffy antigen in parasite invasion has been elucidated in considerable detail.

The Duffy antigen receptor for chemokines—Duffy blood group is determined by two co-dominant alleles, *FY*1* and *FY*2*, which encode respectively the Fy^a and FY^b blood group antigens. The population expression of these antigens is regionally specific and is determined by mutations affecting these alleles that give rise to four major phenotypes: Fy(a+b+), Fy(a+b−), Fy(a-b+) and Fy(a−b−) [4]. In the absence of an obvious disadvantage of Duffy negativity on RBC, the precise role of the Duffy antigen has been something of a mystery. However, it has now been shown that Duffy binds a wide range of pro-inflammatory chemokines, leading to the hypothesis that it may have an important role in modulating their concentrations in plasma [5] and to the new name, Duffy antigen receptor for chemokines (DARC). Further interest in DARC has been fuelled by its potential role in asthma [6], in susceptibility and survival from HIV [7••] and as a determinant of peripheral blood neutrophil counts [8]. Nevertheless, our focus here will be on recent developments regarding the involvement of DARC in malaria biology.

Duffy antigen receptor for chemokines negativity supports the importance of *Plasmodium vivax* malaria—The Fy(a−b−) Duffy negative phenotype resulting from a GATA-1 mutation in the promoter region of the *DARC* gene [9] has reached fixation in much of west and central Africa and accounts for the absence of *P. vivax* malaria from the region [3]. This conclusion is reinforced by a recent survey conducted using sensitive PCR-based typing methods, which found no occurrences of *P. vivax* malaria in nine African malaria-endemic countries [10]. Given the widespread perception of *P. vivax* malaria as a ‘benign’ disease, the reason that DARC negativity has reached such high frequencies has been the subject of some speculation. However, two recent studies conducted in Indonesia [11••] and in Papua New Guinea (PNG) [12••], have challenged this perception and suggest that *P. vivax* might well have exerted a selective pressure, particularly in the era prior to the development of effective treatments.

Duffy antigen receptor for chemokines and *Plasmodium vivax* infection—Although the link between DARC negativity and *P. vivax* resistance was originally made in Africa, much of the subsequent work has been undertaken in areas where *P. vivax* remains a significant clinical problem. Of particular interest are studies conducted by a group working in PNG. In the late 1990s, they identified a small number of heterozygotes for a new Duffy negative allele, Fy^*A^{null}, which was associated with 50% lower Fy^a expression [13]. Subsequent studies have confirmed that such persons are half as likely to be infected with *P. vivax* at cross-sectional survey, are significantly less likely to suffer from clinical *P. vivax* infections, and, if they are infected, parasite densities in them are significantly lower than those achieved in their normal counterparts [14•], observations that confirm the importance of DARC expression in *P. vivax* infections.

The mechanism of RBC invasion of *Plasmodium vivax* merozoites— Recognizing the importance of DARC to *P. vivax* transmission has led to a detailed understanding of the molecular mechanisms by which *P. vivax* invades RBC (Fig. 2) [15]. In recent years, it has been shown that this involves a complex, multistep process, one of which is critically dependent on a specific molecular interaction between DARC, expressed on the RBC surface, and the *P. vivax* Duffy-binding protein (PvDBP) secreted from the micronemes of the *P. vivax* merozoite (reviewed by Chitnis and Sharma [16]). The binding site for PvDBP maps to a 35 amino acid sequence at the N-terminal extracellular region of DARC, whereas the receptor-binding domain lies in cysteine-rich region II of the PvDBP (PvDBPII) [17]. Because this interaction is so critical to the invasion of *P. vivax* into RBC, it has been the focus of considerable scientific interest. Two recent studies have been particularly informative. First, Grimberg *et al.* [18••] used recombinant proteins based on PvDBPII (rPvDBPII) to generate antibodies that inhibited binding of rPvDBPII to DARC. Further, these antibodies bound to native PvDBPII and reduced the ex-vivo invasion of *P. vivax* parasites into DARC-positive RBC. More recently, the same group has found a strong negative correlation between the presence of natural antibodies that inhibit the binding of PvDBPII to DARC and *P. vivax* infections [19••]. Both studies, therefore, suggest that a vaccine based on PvDBPII might well prove successful, and progress toward this aim is ongoing [16]. Nevertheless, although PvDBP is semiconserved, genetic variability is greatest at the receptor-binding domain, which may complicate both the design and testing of a DBP vaccine [20•,21].

***Plasmodium vivax* infections in Duffy antigen receptor for chemokine-negative patients—**Although the evolution of DARC negativity in PNG suggests human adaptation to *P. vivax*, a number of recent reports suggest that the parasite might also be adapting to its human host. First, in western Kenya, Ryan *et al.* [22] identified parasites with the characteristics of *P. vivax* both in Anopheles mosquitoes and a number of DARC-negative humans. More recently, *P. vivax* parasites have also been identified in a small number of DARC-negative people in the Brazilian Amazon [23,24]. Although these reports suggest, therefore, that *P. vivax* might be able to escape its dependence on DARC and use alternative receptors for invasion, they involved very few participants and the relevance of this finding to the development of a *P. vivax* vaccine remains to be seen.

The ABO blood group

ABO and malaria have both been studied for over 100 years, and there are numerous papers on the effects of ABO blood group on various forms of malaria from multiple countries, many coming to contradictory conclusions (covered in some recent reviews [25,26]). Remarkably, until recently, there has been no clear answer to the crucial and obvious question: does ABO blood group affect susceptibility to life-threatening malaria? Preliminary evidence suggested that blood group A might be detrimental [27,28] and group O protective [29]; however, a definitive case–control study taking into account other, potentially confounding, malaria risk factors such as hemoglobin variants was lacking. This need has now been met by two recent studies agreeing in their conclusions that blood group O confers resistance to severe malaria [30••,31••].

ABO blood group, parasite rosetting and malaria susceptibility—Rosetting is characterized by the binding of *P. falciparum*-infected RBCs to uninfected RBCs to form clusters of cells that are thought to contribute to the pathology of falciparum malaria by obstructing blood flow in small blood vessels [1,32] (Fig. 3). The rosetting phenotype varies between parasite isolates and correlates with severe falciparum malaria in sub-Saharan Africa (reviewed in [1]). Previous work had shown that rosetting parasites form larger, stronger rosettes in non-O blood groups (A, B or AB) than in group O RBCs [33,34].

Furthermore, the percentage of infected RBCs forming rosettes is significantly lower in fresh clinical isolates derived from group O than in non-O patients [35]. It appears that this is because the A and B antigens are receptors for rosetting on uninfected RBCs [36], being bound by a parasite protein called PfEMP1 which is expressed on the surface of infected RBCs [37]. Rosettes still form in group O RBCs (albeit smaller and weaker than in non-O RBC) through the involvement of other RBC molecules which act as alternative receptors for rosetting (see complement receptor 1 and the Knops blood group system below).

Thus, it was reasoned that if rosetting contributes directly to the pathogenesis of severe malaria and is reduced in blood group O RBCs, then group O individuals should be protected against life-threatening malaria. This hypothesis has recently been confirmed through a case–control study conducted in Mali, west Africa, in which the odds ratio (OR) for severe malaria in blood group O versus non-O participants was 0.34 [95% confidence interval (CI) 0.19–0.61, $P < 0.0005$] [30••]. Furthermore, a significant interaction was found between parasite rosette frequency and host ABO blood group, supporting the hypothesis that protection was mediated by reduced rosette formation in group O RBC. This work illustrates how a study of blood group polymorphisms can yield insights into malaria pathogenesis; in this case, providing strong support for the theory that rosetting is important in the pathogenesis of severe malaria.

ABO genotypes and malaria susceptibility—A second recent study on ABO and malaria in sub-Saharan Africa used case–control and family-based association methods to show that non-O alleles are associated with an increased risk of severe malaria (OR 1.18, 95% CI 1.11–1.26, $P < 0.0005$ for all data pooled). The study by Fry *et al.* [31••], which examined *ABO* genotypes [based on four single-nucleotide polymorphisms (SNPs) in the ABO glycosyltransferase gene], rather than serological phenotypes, involved almost 4000 cases of severe malaria from Kenya, The Gambia and Malawi. Using blood group phenotypes inferred from SNP haplotypes, individuals with blood groups A or AB were found to be at particular risk of severe malaria [OR 1.33 (95% CI 1.13–1.56, $P = 0.00065$) and OR 1.59 (95% CI 1.15–2.21, $P = 0.006$) respectively]. Another recent study on a Gambian population confirmed that non-O alleles increase risk of severe malaria (OR 1.26, 95% CI 1.11–1.44, $P = 0.0005$) [38••]. By comparison, when the results of Rowe *et al.* [30••] are presented in a similar form, the Mali study showed an OR of 2.94 (95% CI 1.64–5.26, $P < 0.0005$) for severe malaria in non-O versus O blood groups. The exact magnitude of the protective effect of group O may vary between populations due to regional differences in the prevalence of other malaria-resistance genes, varying levels of malaria transmission and population immunity, and possibly differences in pathogenic mechanisms.

The study of Fry *et al.* [31••] also identified a possible ‘parent of origin effect’ in which non-O alleles inherited from the mother led to a greater risk of severe malaria than non-O alleles inherited from the father. Although the explanation for this remains unclear, a genomic imprinting effect has been suggested.

Implications of studies on ABO and malaria susceptibility—Taken together, the study by Rowe *et al.* [30••], with its focus on pathogenic mechanisms, and that by Fry *et al.* [31••], with its focus on genetic mechanisms, provide strong evidence that individuals with non-O blood groups are at increased risk of severe malaria. Furthermore, the data obtained support the hypothesis that malaria parasite rosetting plays a direct role in the pathogenesis of severe malaria and provide extra impetus for research exploring the potential for rosette-disrupting drugs [39,40] or vaccines [41] as interventions against life-threatening malaria [1]. These results could also have implications for the use of blood transfusions in severe malaria. It is possible that transfusion of non-O blood could promote rosetting; therefore, for

those severe malaria patients requiring blood transfusion, it might be preferable to use group O blood whenever possible.

Blood group O occurs in approximately 40–80% of the population in different parts of Africa (reviewed by [26]). If group O protects against life-threatening malaria, why then is the frequency of O not higher in malarious countries? It seems likely that other balancing selection pressures need to be considered. For example, cholera and other diarrhoeal diseases that may be substantial causes of death in sub-Saharan Africa are more common or more severe in group O individuals [42,43]. Population frequencies of blood group O may, therefore, be determined by the regionally specific selection pressures, with *P. falciparum* playing an important but not exclusive role.

The Knops blood group

The Knops (KN) blood group system consists of nine antigens: the antithetical pairs Kn^a/Kn^b (KN1/KN2), McC^a/McC^b (KN3/KN6) and Sl1/Sl2 (KN4/KN7), as well as the Yk^a (KN5), Sl3 (KN8) and KCAM (KN9) antigens [44,45]. These antigens are located on the complement receptor 1 (CR1) molecule [46–48]. CR1 is a RBC membrane glycoprotein that is important for the removal of immune complexes coated with activated complement components (C3b/C4b) and for the control of complement-activating enzymes [49].

The Helgeson phenotype, parasite rosetting and malaria susceptibility

A role for CR1 in malaria was first suggested by Rowe *et al.* [50], who screened a panel of RBCs with null blood group phenotypes for their ability to form rosettes with *P. falciparum*-infected RBCs. It was found that Helgeson RBCs (the null phenotype for the Knops system) showed greatly reduced rosetting. Furthermore, soluble CR1 protein inhibited rosetting [50] and a mAb specific for the C3b-binding site on CR1 reduced rosetting in both laboratory strains and field isolates [51].

Following the same reasoning as that described above for blood group O, it was hypothesized that if the Helgeson phenotype reduces rosetting and rosetting contributes to the development of life-threatening malaria, then individuals with the Helgeson phenotype should be protected from severe disease. A high frequency of the Helgeson phenotype had been reported in PNG [52], therefore, the association between CR1 levels and malaria susceptibility was examined there. Previous work had shown that the expression of CR1 on RBCs of healthy individuals can vary in the range of 50–1200 molecules per cell [53], and that Helgeson phenotype RBCs usually have fewer than 100 molecules per RBC [54]. In Caucasian, Asian and Melanesian populations, the variation in RBC CR1 levels is known to be genetically determined and is associated with SNPs in intron 27 and exon 22 of the CR1 gene giving low (L) and high (H) expression alleles [53,55,56]. These genetic markers were used to examine the relationship between CR1 levels and malaria susceptibility in a case–control study in a highly malarious region of PNG. Low CR1 levels were found to be extremely common (~80% of the population had <200 CR1 molecules per RBC) and the L allele conferred significant protection against severe malaria in heterozygotes (OR 0.33, 95% CI 0.14–0.77, $P = 0.01$) [56]. LL homozygotes showed a trend towards protection, but this was not statistically significant.

A conflicting result was obtained from a case–control study in Thailand, which showed that LL homozygotes are at increased risk of severe malaria (OR 2.74, 95% CI 1.33–5.66) [57]. Rosetting is not associated with severe malaria in this region (where severe malaria differs in demographic and clinical features compared with sub-Saharan Africa) (discussed in [1]). A second study in Thailand found no effect of the intron 27 SNP on susceptibility to severe or cerebral malaria [58]. A range of other SNPs within the CR1 coding sequence [55,59]

also had no association with malaria susceptibility [58]; however, a promoter polymorphism associated with low CR1 expression was a significant risk factor for cerebral malaria [58].

A possible explanation for some of the discrepancies between studies was provided by a recent study from India, which reported that the effects of RBC CR1 expression level vary depending on malaria endemicity. In a population with low/epidemic malaria transmission, there was a significant correlation between low CR1 expression and severe malaria; however, in a higher transmission area, high CR1 levels were associated with disease [60••]. It may be that different pathogenic mechanisms are operating in regions of varying endemicity and in different disease states, with rosetting being an important factor in some circumstances, whereas other factors such as ability to remove immune-complexes could be important in others [61•,62•].

Knops antigens and malaria susceptibility in sub-Saharan Africa

The effect of the Knops blood group system in sub-Saharan Africa is not well understood. An initial study in The Gambia reported no significant association between the CR1 low expression allele (L) and susceptibility to severe malaria [63]; however, it was later realized that the L allele does not correlate with RBC CR1 expression level in African populations [64,65].

One remarkable feature of CR1 in Africa is the occurrence at high frequency of the Knops antigens Sl2 and McC^b (Table 1) [47,66,67]. The *Sl2* SNP is under positive selection [68•], and it has been hypothesized that *Sl2* may offer a survival advantage in a malaria-endemic setting. In-vitro studies showed that adhesion to the malaria parasite rosette-forming protein PfEMP1 expressed in COS-7 cells was impaired in RBCs displaying the Sl:-1,2 [previously called Sl(a–)] phenotype [50]. This implies that Sl:-1,2 RBCs will show reduced rosetting with *P. falciparum*-infected RBCs; however, this has not yet been demonstrated. The effect of the *Sl2* and *McC^b* alleles on malaria susceptibility has been examined in three studies to date, with conflicting results. A case–control study in The Gambia reported no significant association between the *Sl2* or *McC^b* alleles and protection from severe malaria [66]. In contrast, a study in western Kenya found that children with the *Sl2/Sl2* genotype were at reduced risk of cerebral malaria (OR 0.17, 95% CI 0.04–0.72, $P = 0.02$) compared with children with *Sl1/Sl1* [67]. A recent study of a Gambian population found no significant effect of the *Sl2* SNP (rs17047661) on malaria susceptibility [38••].

The differences between these studies may be explained by ethnic differences, study design, varying pathogenic mechanisms in different areas relating to transmission intensity, levels of immunity, or interactions with other malaria-resistance genes. These contradictory reports demonstrate that the role of CR1 in malaria pathogenesis is not yet clearly understood and that more studies involving larger samples and more diverse populations are needed.

Other blood groups and malaria: unanswered questions

The Gerbich negative phenotype (caused by a deletion in the gene encoding Glycophorin C, *GYPC*) is common in malarious regions of PNG [69], and Glycophorin C is an RBC receptor for *P. falciparum* invasion [70,71]. Although it is plausible, therefore, to suggest that in PNG, Gerbich negativity may have been selected to its current frequencies through a survival advantage against severe malaria, this has not been formally tested. Glycophorin B is also an invasion receptor [72•], and again, the effect of Glycophorin B negativity (the S-s-U- phenotype, found almost exclusively in people of African ancestry) on malaria susceptibility is unknown. Similarly, although other blood group antigens that occur at high frequency in malaria-endemic areas, such as the Js^a and V antigens of the KEL and RH

blood group systems, respectively [73], might play a role in malaria resistance, this has not been formally investigated.

Conclusion

Thus, although recent advances in understanding interactions between malaria parasites and the Duffy, ABO and Knops systems illustrate that much has already been learned, it is clear that many intriguing possibilities remain to be explored. Future studies of blood group antigens will continue to provide valuable insights into human malaria.

Acknowledgments

We are grateful to Monica Arman for Figs 1 and 3 and to James Beeson and Brendan Crabb for Fig. 2. We are also grateful to our colleagues in Edinburgh, Mali, Kenya and the United States for discussions on this topic. Our work is funded by the Wellcome Trust (grant no. WT084226 to J.A.R. and WT076934 to T.N.W.).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 527–528).

1. Rowe JA, Claessens A, Corrigan RA, et al. Adhesion of *Plasmodium falciparum*-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. Expert Rev Mol Med. 2009; 11:e16. [PubMed: 19467172]
2. Miller LH, Mason SJ, Dvorak JA, et al. Erythrocyte receptors for (*Plasmodium knowlesi*) malaria: Duffy blood group determinants. Science. 1975; 189:561–563. [PubMed: 1145213]
3. Miller LH, Mason SJ, Clyde DF, et al. The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood- group genotype, FyFy. N Engl J Med. 1976; 295:302–304. [PubMed: 778616]
4. Tournamille C, Le Van Kim C, Gane P, et al. Molecular basis and PCR-DNA typing of the Fya/Fyb blood group polymorphism. Hum Genet. 1995; 95:407–410. [PubMed: 7705836]
5. Fukuma N, Akimitsu N, Hamamoto H, et al. **A** role of the Duffy antigen for the maintenance of plasma chemokine concentrations. Biochem Biophys Res Commun. 2003; 303:137–139. [PubMed: 12646177]
6. Vergara C, Tsai YJ, Grant AV, et al. Gene encoding Duffy antigen/receptor for chemokines is associated with asthma and IgE in three populations. Am J Respir Crit Care Med. 2008; 178:1017–1022. [PubMed: 18827265]
7••. He W, Neil S, Kulkarni H, et al. Duffy antigen receptor for chemokines mediates trans-infection of HIV-1 from red blood cells to target cells and affects HIV-AIDS susceptibility. Cell Host Microbe. 2008; 4:52–62. [PubMed: 18621010] [Pro-inflammatory cytokines have long been recognized as important modifiers of HIV infection. This paper provides the first evidence to suggest that DARC, which is itself an important modulator of plasma cytokine levels, at once increases susceptibility to HIV-1 infection while attenuating the course of HIV disease. This study, therefore, introduces the fascinating possibility that a second infectious disease may be exerting selective pressure on the *DARC* locus.]
8. Reich D, Nalls MA, Kao WH, et al. Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene. PLoS Genet. 2009; 5:e1000360. [PubMed: 19180233]

9. Tournamille C, Colin Y, Cartron JP, et al. Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in Duffy-negative individuals. Nat Genet. 1995; 10:224–228. [PubMed: 7663520]

10. Culleton RL, Mita T, Ndounga M, et al. Failure to detect *Plasmodium vivax* in West and Central Africa by PCR species typing. Malar J. 2008; 7:174. [PubMed: 18783630]

11••. Tjitra E, Anstey NM, Sugiarto P, et al. Multidrug-resistant *Plasmodium vivax* associated with severe and fatal malaria: a prospective study in Papua, Indonesia. PLoS Med. 2008; 5:e128. [PubMed: 18563962] [*P. vivax* is responsible for a considerable proportion of the world's malaria burden. Nevertheless, compared with *P. falciparum*, the disease has been somewhat neglected because traditionally it has been considered benign. This paper provides detailed epidemiological data that challenge this view. 'Severe malaria' was common among children presenting with *P. vivax* malaria and in many ways comparable with *P. falciparum* disease. This paper suggests that in days gone by, before effective treatments were available, *P. vivax* may well have been associated with considerable mortality, an important consideration when interpreting the current distribution of DARC negativity.]

12••. Genton B, D'Acremont V, Rare L, et al. *Plasmodium vivax* and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. PLoS Med. 2008; 5:e127. [PubMed: 18563961] [This paper, simultaneously published in the same volume of *PLoS Medicine* with Ref. [11], also, provides detailed epidemiological data that challenges the benign status of *P. vivax* malaria.]

13. Zimmerman PA, Woolley I, Masinde GL, et al. Emergence of FY A(null) in a *Plasmodium vivax*-endemic region of Papua New Guinea. Proc Natl Acad Sci U S A. 1999; 96:13973–13977. [PubMed: 10570183]

14•. Kasehagen LJ, Mueller I, Kiniboro B, et al. Reduced *Plasmodium vivax* erythrocyte infection in PNG Duffy-negative heterozygotes. PLoS ONE. 2007; 2:e336. [PubMed: 17389925] [This paper provides important data proving that heterozygotes for the *DARC*-negative allele, previously identified in the same study population by the authors, have an intermediate risk of *P. vivax* malaria compared with wild-type homozygotes. This study provides the first data regarding the effect of heterozygosity on *P. vivax* risk.]

15. Beeson JG, Crabb BS. Towards a vaccine against *Plasmodium vivax* malaria. PLoS Med. 2007; 4:e350. [PubMed: 18092888]

16. Chitnis CE, Sharma A. Targeting the *Plasmodium vivax* Duffy-binding protein. Trends Parasitol. 2008; 24:29–34. [PubMed: 18023618]

17. Chitnis CE, Miller LH. Identification of the erythrocyte binding domains of *Plasmodium vivax* and *Plasmodium knowlesi* proteins involved in erythrocyte invasion. J Exp Med. 1994; 180:497–506. [PubMed: 8046329]

18••. Grimberg BT, Udomsangpetch R, Xainli J, et al. *Plasmodium vivax* invasion of human erythrocytes inhibited by antibodies directed against the Duffy binding protein. PLoS Med. 2007; 4:e337. [PubMed: 18092885] [This seminal paper shows that antibodies directed towards PvDBPII inhibit binding of PvDBP to DARC in three different systems and also inhibit invasion *in vitro* by clinical *P. vivax* isolates. These results provide strong support for the development of a *P. vivax* vaccine directed towards PvDBPII.]

19••. King CL, Michon P, Shakri AR, et al. Naturally acquired Duffy-binding protein-specific binding inhibitory antibodies confer protection from blood-stage *Plasmodium vivax* infection. Proc Natl Acad Sci U S A. 2008; 105:8363–8368. [PubMed: 18523022] [This study provides the best evidence to date that naturally acquired antibodies to PvDBP are associated with clinical immunity to *P. vivax* disease.]

20•. Ntumngia FB, McHenry AM, Barnwell JW, et al. Genetic variation among *Plasmodium vivax* isolates adapted to nonhuman primates and the implication for vaccine development. Am J Trop Med Hyg. 2009; 80:218–227. [PubMed: 19190217] [A study showing genetic variability in the receptor-binding domain of the *P. vivax* Duffy binding protein, with important implications for vaccine development and testing.]

21. Gosi P, Khusmith S, Khalambaheti T, et al. Polymorphism patterns in Duffy-binding protein among Thai *Plasmodium vivax* isolates. Malar J. 2008; 7:112. [PubMed: 18582360]

22. Ryan JR, Stoute JA, Amon J, et al. Evidence for transmission of *Plasmodium vivax* among a Duffy antigen negative population in Western Kenya. Am J Trop Med Hyg. 2006; 75:575–581. [PubMed: 17038676]
23. Cavasini CE, de Mattos LC, Couto AA, et al. Duffy blood group gene polymorphisms among malaria vivax patients in four areas of the Brazilian Amazon region. Malar J. 2007; 6:167. [PubMed: 18093292]
24. Cavasini CE, Mattos LC, Couto AA, et al. *Plasmodium vivax* infection among Duffy antigen-negative individuals from the Brazilian Amazon region: an exception? Trans R Soc Trop Med Hyg. 2007; 101:1042–1044. [PubMed: 17604067]
25. Uneke CJ. Plasmodium falciparum malaria and ABO blood group: is there any relationship? Parasitol Res. 2007; 100:759–765. [PubMed: 17047997]
26. Cserti CM, Dzik WH. The ABO blood group system and *Plasmodium falciparum* malaria. Blood. 2007; 110:2250–2258. [PubMed: 17502454]
27. Fischer PR, Boone P. Short report: severe malaria associated with blood group. Am J Trop Med Hyg. 1998; 58:122–123. [PubMed: 9452303]
28. Lell B, May J, Schmidt-Ott RJ, et al. The role of red blood cell polymorphisms in resistance and susceptibility to malaria. Clin Infect Dis. 1999; 28:794–799. [PubMed: 10825041]
29. Pathirana SL, Alles HK, Bandara S, et al. ABO-blood-group types and protection against severe, *Plasmodium falciparum* malaria. Ann Trop Med Parasitol. 2005; 99:119–124. [PubMed: 15814030]
30••. Rowe JA, Handel IG, Thera MA, et al. Blood group O protects against severe *Plasmodium falciparum* malaria through the mechanism of reduced rosetting. Proc Natl Acad Sci U S A. 2007; 104:17471–17476. [PubMed: 17959777] [A case–control study in West Africa that shows the protective effect of group O and provides strong evidence that *P. falciparum* rosetting contributes to malaria pathogenesis. Together with the paper below, this work settles the controversy over the effect of ABO blood group on susceptibility to life-threatening malaria.]
31••. Fry AE, Griffiths MJ, Auburn S, et al. Common variation in the ABO glycosyl-transferase is associated with susceptibility to severe *Plasmodium falciparum* malaria. Hum Mol Genet. 2008; 17:567–576. [PubMed: 18003641] [Case–control and family-based association studies that show that non-O alleles are risk factors for severe malaria across multiple sub-Saharan countries and suggest a possible parent of origin effect.]
32. Kaul DK, Roth EFJ, Nagel RL, et al. Rosetting of *Plasmodium falciparum*-infected red blood cells with uninfected red blood cells enhances micro-vascular obstruction under flow conditions. Blood. 1991; 78:812–819. [PubMed: 1859893]
33. Carlson J, Wahlgren M. *Plasmodium falciparum* erythrocyte rosetting is mediated by promiscuous lectin-like interactions. J Exp Med. 1992; 176:1311–1317. [PubMed: 1402677]
34. Udomsangpetch R, Todd J, Carlson J, et al. The effects of hemoglobin genotype and ABO blood group on the formation of rosettes by *Plasmodium falciparum*-infected red blood cells. Am J Trop Med Hyg. 1993; 48:149–153. [PubMed: 8447516]
35. Rowe A, Obeiro J, Newbold CI, et al. *Plasmodium falciparum* rosetting is associated with malaria severity in Kenya. Infect Immun. 1995; 63:2323–2326. [PubMed: 7768616]
36. Barragan A, Kremsner PG, Wahlgren M, et al. Blood group A antigen is a coreceptor in *Plasmodium falciparum* rosetting. Infect Immun. 2000; 68:2971–2975. [PubMed: 10768996]
37. Chen Q, Heddini A, Barragan A, et al. The semiconserved head structure of *Plasmodium falciparum* erythrocyte membrane protein 1 mediates binding to multiple independent host receptors. J Exp Med. 2000; 192:1–10. [PubMed: 10880521]
38••. Jallow M, Teo YY, Small KS, et al. Genome-wide and fine-resolution association analysis of malaria in West Africa. Nat Genet. 2009; 41:657–665. [PubMed: 19465909] [A broad-reaching analysis of the ability of a genome wide association (GWA) study to detect malaria resistance genes in an African population. This paper identifies problems with traditional GWA approaches in African populations and puts forward strategies to deal with them. Known malaria-resistance genes are also examined in detail.]
39. Vogt AM, Pettersson F, Moll K, et al. Release of sequestered malaria parasites upon injection of a glycosaminoglycan. PLoS Pathog. 2006; 2:e100. [PubMed: 17009869]

40. Kyriacou HM, Steen KE, Raza A, et al. In vitro inhibition of *Plasmodium falciparum* rosette formation by Curdlan sulfate. Antimicrob Agents Chemother. 2007; 51:1321–1326. [PubMed: 17283200]
41. Chen Q, Pettersson F, Vogt AM, et al. Immunization with PfEMP1-DBL1alpha generates antibodies that disrupt rosettes and protect against the sequestration of *Plasmodium falciparum*-infected erythrocytes. Vaccine. 2004; 22:2701–2712. [PubMed: 15246600]
42. Swerdlow DL, Mintz ED, Rodriguez M, et al. Severe life-threatening cholera associated with blood group O in Peru: implications for the Latin American epidemic. J Infect Dis. 1994; 170:468–472. [PubMed: 8035040]
43. Black RE, Levine MM, Clements ML, et al. Association between O blood group and occurrence and severity of diarrhoea due to *Escherichia coli*. Trans R Soc Trop Med Hyg. 1987; 81:120–123. [PubMed: 3127953]
44. Daniels G, Flegel WA, Fletcher A, et al. International Society of Blood Transfusion Committee on Terminology for Red Cell Surface Antigens: Cape Town report. Vox Sang. 2007; 92:250–253. [PubMed: 17348875]
45. Daniels GL, Fletcher A, Garratty G, et al. Blood group terminology 2004: from the International Society of Blood Transfusion committee on terminology for red cell surface antigens. Vox Sang. 2004; 87:304–316. [PubMed: 15585029]
46. Moulds JM, Nickells MW, Moulds JJ, et al. The C3b/C4b receptor is recognised by the Knops, McCoy, Swain-Langley and York blood group antisera. J Exp Med. 1991; 173:1159–1163. [PubMed: 1708809]
47. Moulds JM, Zimmerman PA, Doumbo OK, et al. Molecular identification of Knops blood group polymorphisms found in long homologous region D of complement receptor 1. Blood. 2001; 97:2879–2885. [PubMed: 11313284]
48. Tamasauskas D, Powell V, Schawalder A, et al. Localization of Knops system antigens in the long homologous repeats of complement receptor 1. Transfusion. 2001; 41:1397–1404. [PubMed: 11724985]
49. Khera R, Das N. Complement receptor 1: disease associations and therapeutic implications. Mol Immunol. 2008; 46:761–772. [PubMed: 19004497]
50. Rowe JA, Moulds JM, Newbold CI, et al. *P. falciparum* rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1. Nature. 1997:388–292.
51. Rowe JA, Rogerson SJ, Raza A, et al. Mapping of the region of complement receptor (CR) 1 required for *Plasmodium falciparum* rosetting and demonstration of the importance of CR1 in rosetting in field isolates. J Immunol. 2000; 165:6341–6346. [PubMed: 11086071]
52. Molthan L. The status of the McCoy/Knops antigens. Med Lab Sci. 1983; 40:59–63. [PubMed: 6865671]
53. Wilson JG, Murphy EE, Wong WW, et al. Identification of a restriction fragment length polymorphism by a CR1 cDNA that correlates with the number of CR1 on erythrocytes. J Exp Med. 1986; 164:50–59. [PubMed: 3014040]
54. Moulds JM, Moulds JJ, Brown M, et al. Antiglobulin testing for CR1-related (Knops/McCoy/Swain-Langley/York) blood group antigens: negative and weak reactions are caused by variable expression of CR1. Vox Sang. 1992; 62:230–235. [PubMed: 1379395]
55. Xiang L, Rundles JR, Hamilton DR, et al. Quantitative alleles of CR1: coding sequence analysis and comparison of haplotypes in two ethnic groups. J Immunol. 1999; 163:4939–4945. [PubMed: 10528197]
56. Cockburn IA, Mackinnon MJ, O'Donnell A, et al. A human complement receptor 1 polymorphism that reduces *Plasmodium falciparum* rosetting confers protection against severe malaria. Proc Natl Acad Sci U S A. 2004; 101:272–277. [PubMed: 14694201]
57. Nagayasu E, Ito M, Akaki M, et al. CR1 density polymorphism on erythrocytes of falciparum malaria patients in Thailand. Am J Trop Med Hyg. 2001; 64:1–5. [PubMed: 11425154]
58. Teeranaipong P, Ohashi J, Patarapotikul J, et al. A functional single-nucleotide polymorphism in the CR1 promoter region contributes to protection against cerebral malaria. J Infect Dis. 2008; 198:1880–1891. [PubMed: 18954261]

59. Thomas BN, Donvito B, Cockburn I, et al. A complement receptor-1 polymorphism with high frequency in malaria endemic regions of Asia but not Africa. Genes Immun. 2005; 6:31–36. [PubMed: 15578041]

60••. Sinha S, Jha GN, Anand P, et al. CR1 levels and gene polymorphisms exhibit differential association with falciparum malaria in regions of varying disease endemicity. Hum Immunol. 2009; 70:244–250. [PubMed: 19480840] [A paper that shows that the effect of RBC CR1 levels on malaria susceptibility varies in regions of differing malaria transmission intensity. This work is important in providing a plausible explanation for previous conflicting results from different regions.]

61•. Owuor BO, Odhiambo CO, Otieno WO, et al. Reduced immune complex binding capacity and increased complement susceptibility of red cells from children with severe malaria-associated anemia. Mol Med. 2008; 14:89–97. [PubMed: 18317566] [Additional recent work emphasizing the possible role of immune complexes in malaria pathogenesis.]

62•. Mibei EK, Otieno WO, Orago AS, et al. Distinct pattern of class and subclass antibodies in immune complexes of children with cerebral malaria and severe malarial anaemia. Parasite Immunol. 2008; 30:334–341. [PubMed: 18466201] [Additional recent work emphasizing the possible role of immune complexes in malaria pathogenesis.]

63. Bellamy R, Kwiatkowski D, Hill AV. Absence of an association between intercellular adhesion molecule 1, complement receptor 1 and interleukin 1 receptor antagonist gene polymorphisms and severe malaria in a West African population. Trans R Soc Trop Med Hyg. 1998; 92:312–316. [PubMed: 9861406]

64. Herrera AH, Xiang L, Martin SG, et al. Analysis of complement receptor type 1 (CR1) expression on erythrocytes and of CR1 allelic markers in Caucasian and African American populations. Clin Immunol Immunopathol. 1998; 87:176–183. [PubMed: 9614933]

65. Rowe JA, Raza A, Diallo DA, et al. Erythrocyte CR1 expression level does not correlate with a HindIII restriction fragment length polymorphism in Africans: implications for studies on malaria susceptibility. Genes Immun. 2002; 3:497–500. [PubMed: 12486610]

66. Zimmerman PA, Fitness J, Moulds JM, et al. CR1 Knops blood group alleles are not associated with severe malaria in the Gambia. Genes Immun. 2003; 4:368–373. [PubMed: 12847553]

67. Thathy V, Moulds JM, Guyah B, et al. Complement receptor 1 polymorphisms associated with resistance to severe malaria in Kenya. Malar J. 2005; 4:54. [PubMed: 16277654]

68•. Barreiro LB, Laval G, Quach H, et al. Natural selection has driven population differentiation in modern humans. Nat Genet. 2008; 40:340–345. [PubMed: 18246066] [This paper is important for providing evidence that the CR1 Knops blood group antigen encoded by the *Sl2* SNP is under positive selection in an African population. Whether this selection is due to malaria remains unclear.]

69. Patel SS, King CL, Mgone CS, et al. Glycophorin C (Gerbich antigen blood group) and band 3 polymorphisms in two malaria holoendemic regions of Papua New Guinea. Am J Hematol. 2004; 75:1–5. [PubMed: 14695625]

70. Maier AG, Duraisingh MT, Reeder JC, et al. *Plasmodium falciparum* erythrocyte invasion through glycophorin C and selection for Gerbich negativity in human populations. Nat Med. 2003; 9:87–92. [PubMed: 12469115]

71. Mayer DC, Jiang L, Achur RN, et al. The glycophorin C N-linked glycan is a critical component of the ligand for the *Plasmodium falciparum* erythrocyte receptor BAEBL. Proc Natl Acad Sci U S A. 2006; 103:2358–2362. [PubMed: 16461900]

72•. Mayer DC, Cofie J, Jiang L, et al. Glycophorin B is the erythrocyte receptor of *Plasmodium falciparum* erythrocyte-binding ligand, EBL-1. Proc Natl Acad Sci U S A. 2009; 106:5348–5352. [PubMed: 19279206] [A study that clarifies the mechanism of a *P. falciparum* invasion pathway and raises the possibility that mutations in the gene encoding Glycophorin B in malaria endemic areas could affect susceptibility to malaria.]

73. Miller LH. Impact of malaria on genetic polymorphism and genetic diseases in Africans and African Americans. Proc Natl Acad Sci USA. 1994; 91:2415–2419. [PubMed: 8146132]

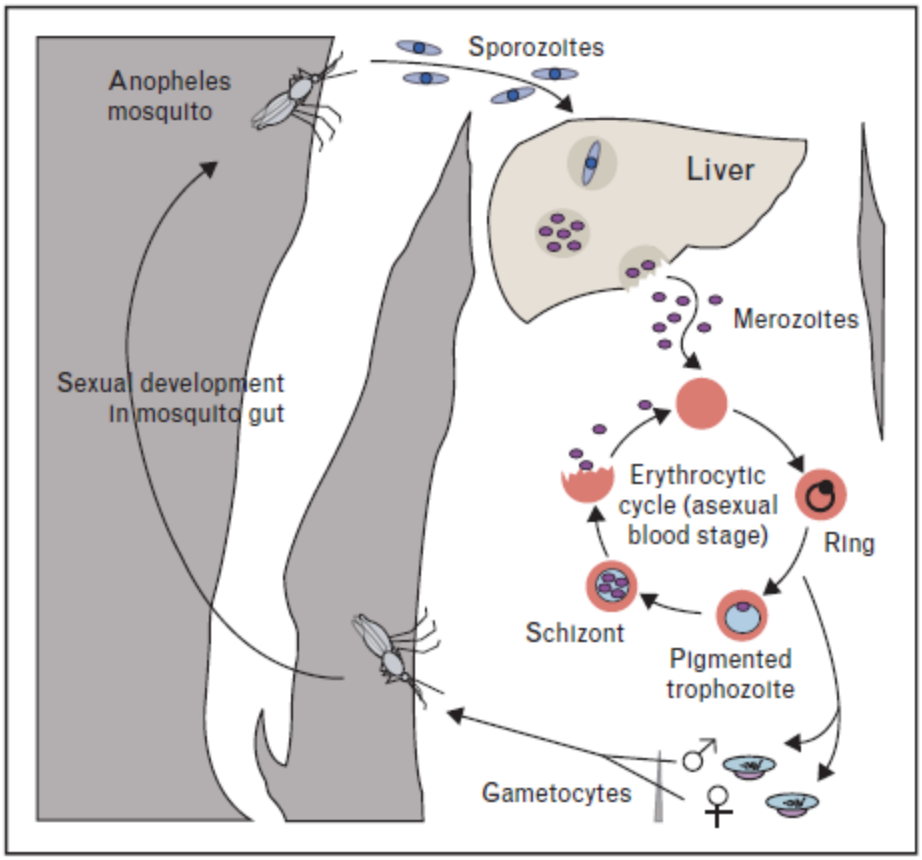

Figure 1. Life cycle of *Plasmodium falciparum*

When an infected female *Anopheles* mosquito takes a blood meal, sporozoite forms of *Plasmodium falciparum* are injected into the human skin. The sporozoites migrate into the bloodstream and then invade liver cells. The parasite grows and divides within liver cells for 8–10 days, then daughter cells, called merozoites, are released from the liver into the bloodstream, where they rapidly invade red blood cells (RBCs). Merozoites subsequently develop into ring, pigmented-trophozoite, and schizont stage parasites within the infected RBC. *P. falciparum*-infected erythrocytes express parasite-derived adhesion molecules on their surface, resulting in sequestration of pigmented-trophozoite and schizont stage-infected RBCs in the microvasculature. The asexual intraerythrocytic cycle lasts 48 h and is completed by the formation and release of new merozoites that will re-invade uninfected RBCs. It is during this asexual bloodstream cycle that the clinical symptoms of malaria (fever, chills, impaired consciousness, etc.) occur. During the asexual cycle, some of the infected RBCs develop into male and female sexual stages called gametocytes that are available to be taken up by feeding female mosquitoes. The gametocytes are fertilized and undergo further development in the mosquito, resulting in the presence of sporozoites in the mosquito's salivary glands ready to infect another human host. Reproduced with permission from [1].

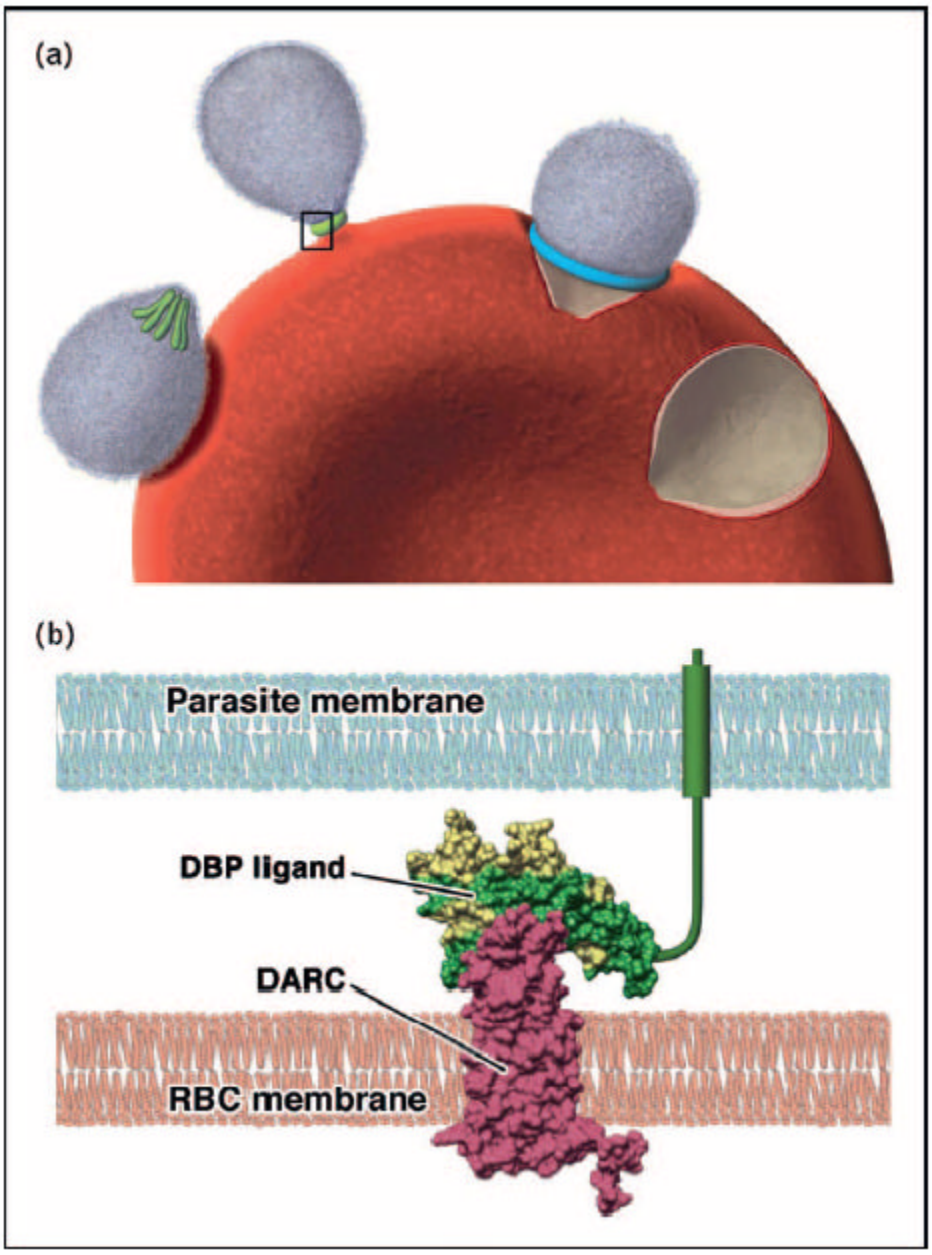

Figure 2. The role of Duffy antigen receptor for chemokines in *Plasmodium vivax* invasion

(a) The *Plasmodium vivax* Duffy binding protein (PvDBP) is located in the micronemes of the merozoite (green). After attachment of the merozoite to the red blood cell (RBC) (reticulocyte) surface, the merozoite re-orientates, so that its apical end is in contact with the RBC membrane. DBP is then released and a tight junction (blue) is formed between the merozoite and the RBC membrane. The tight junction moves from the apical to posterior pole as the merozoite invades the RBC, propelled by an actin-myosin motor. The RBC membrane is resealed once invasion is complete. The entire process from initial attachment to completed invasion takes approximately 1 min. (b) A model of the binding of the PvDBP to the Duffy antigen receptor for chemokines (DARC) [inset from (a)]. Amino acid residues in PvDBP that are conserved are green and polymorphic yellow. Antibodies are predicted to

bind to a polymorphic region of the DBP that is separate from, but may overlap with, the DARC-binding site. Reproduced with permission from [15].

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

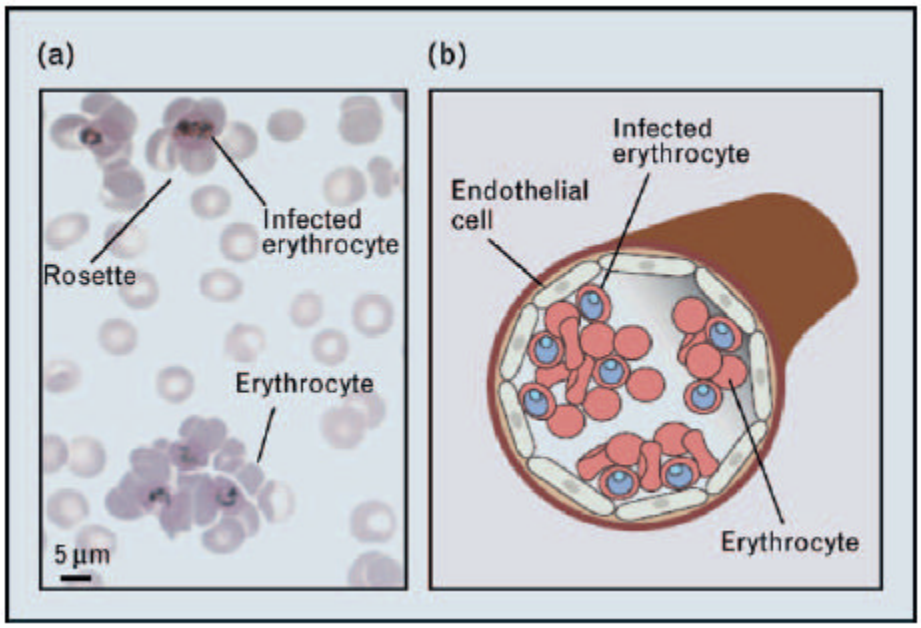

Figure 3. *Plasmodium falciparum* rosetting

(a) Rosetting in a *Plasmodium falciparum* in-vitro culture, observed after preparation of a Giemsa-stained thin smear and light microscopy. (b). Schematic representation of *P. falciparum* rosette formation in the microvasculature. Rosetting infected red blood cells (RBCs) are thought to have the ability to bind simultaneously to microvascular endothelial cells and uninfected RBCs, resulting in obstruction to blood flow in microvessels contributing to pathological effects such as hypoxia and acidosis. Adapted with permission from [1].

Europe PMC Funders Author Manuscripts

Table 1

Percentage of the Knops blood group system *Sl1/Sl2* and McC^a/McC^b genotypes in various populations

Population *(n)* [reference]	*Sl1/Sl1*	*Sl1/Sl2*	*Sl2/Sl2*	McC^a/McC^a	McC^a/McC^b	McC^b/McC^b
Mali (99) [47]	9	30	61	50	40	10
The Gambia (853) [66]	5	30	65	38	47	15
Western Kenya (460) [67]	10	44	45	48	45	7
Caucasian Americans (100) [66]	99	1	0	100	0	0
Asian Americans (99) [66]	95	4	1	96	4	0
Hispanic Americans (100) [66]	94	6	0	95	5	0